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THE STRESS INDUCED VOLATILE EMISSIONS (SIVE) TECHNIQUE FOR MEASURING LEVELS OF STRESS IN CONIFER SEEDLINGS

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ABSTRACT

The stress induced volatile emissions technique (SIVE) uses gas chromatography to measure the levels of certain gases (such as acetaldehyde, ethane, ethanol, or ethylene) which are released in a response to a given stress by plant tissue (needles and stems). This manual is intended to give a short outline of the technique. It provides instructions necessary to sample the tissue, to run the samples through the gas chromatograph (GC) ensuring safe operation at all times, to collect the data for analysis by computer and to pack the two analytical columns utilized in this procedure.

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1.0 GC BASICS

1.1 Overview

Gas chromatography is a technique used for separating mixtures of volatile (gaseous) compounds into discrete packages or fractions. These fractions can then be collected for further analysis or the individual compounds can be tentatively identified by comparison of their retention times (RT) with those of known standards. The retention time is the time taken for a gas to pass through an analytical column. The RT is different for different gases and for different analytical columns.

A mixture of compounds is injected onto a column through which is flowing an inert carrier gas such as helium (He) or nitrogen (N_2). The carrier gas propels the mixture through the column. The column is packed or lined with materials that retard the passage of different components of the mixture to different degrees depending on the chemical and physical properties of the components such as molecular weight, charge or solubility.

As a result, the mixture arrives at the end of the column with its components separated into fractions. The passage of these fractions out of the column can be monitored by a detector. The detector responds to the gas and translates the passage of that gas into an electrical impulse which can be displayed on a printer plotter or manipulated by a computer.

The area under the peak, measured by the detector, is related to the mass of the compound passing the detector. The time from injection of the mixture to the time an individual component passes the detector is known as the retention time (RT) and will remain the same for a particular compound under identical conditions (column size, composition and temperature of the column). The retention time can be used to identify the compound by matching its RT with that of a known sample of the compound.

1.2 Detectors

There are several different types of detectors used in gas chromatography. Three of the most common are: thermal conductivity detectors (TCD), electron capture detectors (ECD) and flame ionization detectors (FID). The SIVE technique employs the flame ionization detector. The FID uses a hydrogen-air flame to ionize the sample as it passes an electrostatic field. The ions are collected at electrodes and this generates a current. The current is proportional to the concentration of the sample. The advantage of the FID detector is that it does not measure O_2, N_2, CO_2 or water. This means it is ideal for detecting hydrocarbons in atmospheric samples and they need not be anhydrous.

1.3 Coldtrapping

Samples used in the SIVE technique are obtained by withdrawing a portion of the headspace gas from a closed volume containing the plant tissue being examined. The headspace is the volume of the container not occupied by the tissue or any liquid present.

A technique for concentrating the gases of interest is necessary because the amounts of gases given off by the plant tissue are very small and the columns used (see Section 2.4 and Appendix II) can only receive a maximum of 2 mL of injected sample. Thus in most instances, the amounts of some of the gases being examined, in an unconcentrated sample, are practically undetectable with a 2 mL injection.

There are two ways to overcome this problem. The first is to incubate the tissue being measured over a long period of time. This allows the concentration of gases of interest to build up to levels high enough to measure when 2 mL or less of the headspace is injected onto the column. The problem with this approach is that incubation may take up to 24 hours. This negates the speed which is the advantage of the SIVE test. Also, there is a possibility that some of the build-up of gases may be the result of prolonged confinement in the incubation container. This is especially true of acetaldehyde and ethanol which may be produced when oxygen in the incubation container becomes limiting (initiation of anaerobic pathways).

The second way to overcome the problem is to take a large volume of the headspace and concentrate it into a 1 mL volume containing only the gases of interest by removing the large volumes of atmospheric gases, N_2, O_2 and CO_2. When the 1 mL volume is injected onto the column, it will be the equivalent of injecting the much larger headspace volume.

This concentration of gases is achieved by passing them through a column that retains the gases of interest at low temperatures but allows N_2, O_2 and CO_2 to pass through. The technique is known as coldtrapping. At high temperatures, the retained gases are then released so that they can be collected and passed through the analytical column.

The trapping column used is a short (16.0 cm) HayeSep N column (see Appendix II). It has been connected to the GC so that a headspace sample can be injected onto the trapping column while the machine is in backflush mode. Then when the GC is in run mode, the carrier gas passes through the cold-trapping column sweeping the concentrated gases onto the analytical column.

When the sample is being loaded onto the coldtrapping column, the temperature has to be kept low. When the sample is to be released, the temperature should be raised to above the boiling point of the highest boiling point component of the gas sample. The temperatures used must be repeatable. The simplest way to reproduce the highest temperature is to immerse the trapping column in a boiling water

bath. The cold temperatures can be any temperature below room temperature. Up to a point, decreased temperature results in increased trapping and hence sample concentration, but decreasing temperatures becomes increasingly costly to achieve. Below about -25°C, increases in trapping efficiency are marginal.

1.3.1 Cold bath

A number of cold baths can be created that will maintain a temperature below zero. A solution of $CaCl_2$ will depress the freezing point of water. The greater the concentration, the greater the freezing point depression until a maximum depression of -49.7°C is achieved at a concentration of 32 percent by weight of $CaCl_2$. In the SIVE technique, a temperature of -22°C has been maintained using a concentration of 29 gm $CaCl_2$ · 100 mL^{-1} H_2O. This temperature can be maintained in an ordinary household type chest freezer.

2.0 OUTLINE OF THE SIVE TECHNIQUE

2.1 Tissue sampling

Seedlings to be sampled have their roots excised at the root collar and the shoot is placed in a 120 mL plastic bottle which has been flushed with hydrocarbon free air. Hydrocarbon free air is obtained by passing ambient air over a potassium permanganate filter. The bottle is then sealed with a serum cap and 120 mL of hydrocarbon free air is immediately flushed through the serum cap via a hypodermic syringe.

The tissue is incubated for 2 hours at room temperature (24°C, a BOD incubator is suitable) and then 50 mL of the headspace gases are drawn off in a syringe as a sample for testing. The removal of the 50 mL sample is compensated for by simultaneously adding 50 mL of hydrocarbon free air. Thus total incubation volume is 170 mL. During sampling, the sample syringe and the clean air syringe are alternately filled and emptied, at least twice, to achieve a mix of their volumes.

After the headspace gas sample is taken, the tissue can be dried in a microwave oven (500 watts). One seedling is given 10 minutes at half power and 2 minutes at full power. Dry weights can then be taken.

2.2 Coldtrapping

50 mL samples of the headspace gases are introduced onto an open-ended, 16.0 X 0.32 cm, coldtrapping column (see Appendix II for packing) in order to remove the nitrogen and oxygen and concentrate the hydrocarbons into a 1 mL injection. The coldtrapping column is packed with HayeSep N. The column is cooled to -20°C with a calcium chloride cold bath. This traps the hydrocarbons on the HayeSep N but allows the N_2 and O_2 to pass through.

Immediately after the experimental sample is introduced onto the coldtrap, the temperature is raised to 100°C using a boiling water bath. This frees the trapped hydrocarbons. Simultaneously, the coldtrap is switched on line with the analytical column of the gas chromatograph and the run is started.

2.3 Gas Chromatography

The gas chromatograph is equipped with a HayeSep Q packed column (180.0 x 0.32 cm) (See Appendix II). The column is backflushed with carrier gas at all times, except during analysis, in order to remove long retention time terpenes from previous runs.

The analysis program starts with the oven and the column inside it at 100°C. This temperature is maintained for 1 min and then is raised to 190 C at a rate of 30°C $\cdot$ min^{-1}. The temperature is again held constant for 2 min. This process takes 8 minutes and constitutes the analytical portion of the run (Appendix I (1. b), Relays 1 and 0, 0.01 - 8.0 min). The column is then switched to backflush mode and the oven temperature raised to 250°C at 30°C $\cdot$ min^{-1}. This final temperature is held for 2 minutes to help remove long retention time terpenes. The run is now complete, and the next run can be started when the oven has cooled down and the starting temperature of 100°C is restored. A complete run from injection to injection takes about 17 minutes.

2.4 Column materials and gas separation

HayeSep porous polymer beads are cross-linked polymers which may be used directly in GC columns. They provide sharp, symmetrical peaks. HayeSep N and Q have different retention properties as a result of various functional groups being incorporated into the porous polymer. HayeSep N is a more polar polymer than HayeSep Q, hence retention properties are different between the two.

The temperature program (Section 2.3 and Appendix I, 1) gives good separation of ethylene and ethane while giving reasonably short retention times for ethanol and acetaldehyde. These are four gases that have been shown to be given off by trees under stress. Ethylene is a plant growth regulator that is believed to be a factor in many types of stress response. Ethane is thought to be produced as the result of the breakdown of cell membranes. Ethanol has been associated with anaerobic stress, but there is evidence that it may be associated with other types of stress. Acetaldehyde is the precursor of ethanol in the synthetic pathway.

The program is currently named "5 modified" and is stored under number 1 in the GC memory.

2.5 Clean air

Hydrocarbon free air is produced by passing ambient air over a Purafil filter (a filter column packed with potassium permanganate coated pellets) using a motor driven air pump.

2.6 Data handling

A hard copy is made of the trace on a printer plotter. Peak areas and retention times, as calculated by the gas chromatograph, are also printed in hard copy as well as being transferred to an IBM compatible computer. The peaks can then be converted to a per dry weight basis and plotted against temperature or PMS using a database program called REFLEX.

3.0 SAMPLING

3.1 Clean air

A supply of hydrocarbon free air is required to make sure that the samples are not contaminated by hydrocarbons in the air, whether from industrial or natural sources.

Clean air is obtained by passing air through a potassium permanganate scrubber. This removes most small, molecular weight hydrocarbons from the air, including those of interest to the SIVE technique.

a) Switch on air pump.

b) Use syringe on outlet hose to fill or flush containers with hydrocarbon free air.

3.2 Standard gases

a) Flush 1 L glass, sample chamber with clean air.

b) Fill a 12 mL test tube, capped with a serum cap, with 1000 ppm ethylene (C_2H_4).

- Insert a 23 gauge needle through the serum cap as an outlet, and the 23 gauge syringe needle attached to a line from the ethylene compressed gas bottle as the inlet.
- Flush for 30 sec at 20 psi.

c) Fill a second 12 mL test tube with 1000 ppm ethane (C_2H_6) as in step b.

d) Half fill a third 12 mL test tube with absolute ethanol and place a serum cap on the tube.

- Flush for 10 seconds with clean air

e) Half fill a fourth 12 mL test tube with acetaldehyde and cap and flush as in step d.

- See Appendix IV - Safety

f) Fill a 1000 microlitre syringe with:

- 100 microlitres of 1000 ppm ethylene,
- 100 microlitres of 1000 ppm ethane,
- 20 microlitres acetaldehyde (headspace gas), and
- 60 microlitres ethanol (headspace gas).

g) Inject this mixture into the clean-air flushed 1 L glass sample chamber via the serum cap on the top.

- Both stopcocks should be closed.

h) Allow standard gases to diffuse (about 5-10 min).

i) Place a 50 mL syringe with an 18 gauge needle into the serum cap in one stopcock.

- This stopcock should now be opened.

j) Fill another 50 ml syringe with clean air (via a 23 gauge needle).

k) Insert the syringe with the 23 gauge needle into the serum cap in the top of the 1 L glass, sample chamber.

l) Alternately, fill and empty the two syringes a minimum of two times to ensure a good mixture.

m) Withdraw the 50 mL syringe (with the 23 gauge needle, see j.) containing a 50 mL sample of the standard gases.

n) Inject into GC as described in Section 5.4.

3.3 Tissue sampling

a) Flush a 120 mL plastic bottle with clean air.

b) Place two, whole seedling shoots into plastic bottle.

- They will fold in if pushed gently.
- CARE must be taken at this stage as this can cause tissue damage and therefore STRESS emission of gases.

c) Close bottle opening with serum cap.

d) Flush closed 120 mL bottle with clean air.

- Place 23 gauge needle in serum cap as outlet.
- Place 23 gauge needle on clean air supply and insert into serum cap.
- Flush for 15 seconds.

e) Allow to incubate.

- 2 hours has been used to date.

f) Using two 50 mL syringes (each with 23 gauge needles attached), one filled with 50 mL of clean air; the other empty, and follow the procedure of 3.2 1-n.

4.0 GC OPERATION

Consult Appendix IV - Safety

The GC used is a Perkin Elmer 8500 and the following instructions are applicable to this GC.

The machine should not be turned off unless it is not going to be used during the next fortnight. Therefore, functions described in this section are usually done ONLY at the start and finish of an experimental series. The column stays cleaner, and less time is lost, day to day, if the machine is left on continuously. The continuous flow of gas keeps the column clean and in good condition for running samples.

Throughout this manual, function keys are used to cause different tasks to be performed depending on the menu displayed. Instructions to press a Function key (e.g. F1) are followed by the relevant menu description.

4.1 Starting the GC

This should be done the night before starting an experiment.

a) Turn on carrier gas (He) at cylinder.

- Gauge on flow regulator should read 100 psi.

b) Turn on compressed air at cylinder.

- Gauge on flow regulator should read 80 psi.

c) Switch on machine.

- Power switch is at lower right hand front.

d) Wait for initialization (indicated on screen).

4.2 Cleaning columns after a shut down

Call up the CLEANUP method in Appendix I.2 and allow to run overnight.

4.3 Restoring screen

The machine has a screen saving function. The screen will go blank if the machine is not in use for a distinct period of time, about 15 min. If this happens, pressing INST/TERM will restore the current image to the screen.

4.4 Turning machine off

This should only be done if the machine is not going to be used for a fortnight.

a) Turn off power (see Section 4.1.c).

b) Turn off all 4 gases (He, H_2, high purity air [UHP], and compressed air [CA]).

5.0 RUNNING SAMPLES

A blank run (no injection) should be done at the start of each day or if a sample has not been run for more than an hour. This is to purge column of any interfering substances.

5.1 Selecting the sample method

After the columns have been cleaned (4.2), install the control program (Appendix I.1) called "5 modified".

5.2 Lighting the flame ionization detector (FID)

This needs to be done at the START of each working day, once the detector is up to operating temperature.

a) Turn on H_2 at cylinder.

- Gauge on flow regulator should read 60 psi.

b) Turn on ultra high purity (UHP) air at cylinder.

- Gauge on flow regulator should read 45 psi.

c) Flip black lever down.

- At top back left of the machine behind the oven.
- Leave down for about 1 min.

d) Touch lighter to detector outlet.

- Lighter wire will glow.

e) Flip black lever into up position.

- Keep glowing lighter on detector outlet.

f) Repeat until flame lights.

- When flame lights, the lighter will glow brighter and there will be a popping sound.
- To further test for a lit flame hold a bright object (a mirror or a chrome plated tool) in front of the detector exit.
- Water condensation indicates a lit flame.

g) Wait for column injector and detector to reach operating temperatures.

h) READY will appear on screen and the run can be initiated.

5.3 Preparing for an injection

Prepare the following mixtures.

a) An ice bath.

b) A boiling water bath in a self-heated pot.

c) A backup source of hot water to top up the boiling water bath.

d) Two -22°C $CaCl_2$ baths kept in freezer compartment.

- $CaCl_2$ solution, 29gm · $100mL^{-1}$ distilled H_2O.
- CRC Handbook of Chemistry and Physics, Section D, concentrative properties of aqueous solutions.

5.4 Making an injection

a) Fill 50 mL syringe (with 23 gauge needle) with 50 mL of hydrocarbon free air.

- See Section 3.1 clean air.

b) Place 23 gauge needle of 50 mL syringe in gas inlet tube and empty syringe through the coldtrap column.

- This will flush the coldtrap column.

c) Immerse the coldtrap column in the ice bath.

d) Fetch the -22°C bath from the freezer compartment.

e) Remove the ice bath and immerse the coldtrap in the -22°C bath.

f) Allow coldtrap to equilibrate for 2 min.

g) Top up the boiling water bath with water from the back up hot water supply.

- Allow time for the water to return to the boil.

h) Introduce 50 mL sample onto coldtrap column in the same manner as the clean air sample in Section 5.4, b.

i) Immediately remove the -22°C bath.

j) Rinse the coldtrap by immersing it in a beaker of clean water.

- Removes the $CaCl_2$ which is corrosive.

k) Immerse the coldtrap into the boiling water bath and allow to equilibrate.

- Time taken to return the -22°C bath to the freezer compartment is adequate, about 60 seconds.

l) Press RUN.

- This will start the data capture and switch the coldtrapping column into the carrier gas line.
- There will be a hiss as the valve switches from backflush to run.

m) When the valve switches to back-flush, preparations can be started for the next sample injection.

- See Section 5.4 (a-f).

n) When the run ends a report will appear on the screen.

5.5 Extinguishing the flame ionization detector

This should be done at the end of each working day.

a) Turn off H_2 at cylinder.

b) Turn off UHP air at cylinder.

5.6 Daily cleanup

a) Turn off all hot plates, kettles, etc.

b) Empty water, ice bath and rinse water containers.

c) Ensure both $CaCl_2$ baths are in freezer compartment.

6.0 TRANSFERRING DATA TO COMPUTER AND TO HARD COPY

6.1 Preparing the computer for data capture

This should be done at the beginning of a work day.

a) Switch on computer.

b) Type D and press ENTER.

c) Put a diskette in drive A.

d) Press ENTER.

- This opens the highlighted window in the multi tasking software DESQVIEW.

e) Select XTALK (Crosstalk XVI 3.6) by moving the highlight with the cursor keys to XTALK.

f) Press ENTER.

- Several "screens" will flash briefly on the screen.

g) When the status line displays "Capture to what file?"

- Type a file name, e.g., A:DATA.GC.
- The file name must have an extension.

h) If this file already exists, the status line will indicate the fact.

- Press A to add to the existing file, if this file is to be used for capturing the data.

i) The computer is ready to receive data when the status line displays:

- "Esc for ATtention, Home to SWitch ¦ Capture to A:DATA.GC ¦ Local".

j) Other software can be selected from DESQVIEW menu to be run at the same time as XTALK.

- ALWAYS load XTALK first.
- WORDPERFECT which can be used for note taking during a run.
- FILE MANAGER to view filename and other directories during a run.
- PROGRAM EDITOR to edit the data gathering program or other programs during a run.

k) Press ALT to retrieve the DESQVIEW menu.

l) Select other programs by moving the highlight to the program name and press ENTER.

m) Change between programs by pressing ALT, then SWITCH WINDOWS, then window #.

- This can be shortened if the window # is known.
- Press ALT, then window #.

n) Use ALT Z to expand a window to fill the screen.

6.2 Transferring data to printer plotter

This must be done at the end of a run before the next run is started because EACH RUN DELETES THE DATA OF THE PREVIOUS RUN FROM THE GC MEMORY.

a) Switch on printer/plotter.

b) Press button A (Printer/plotter) on switch box.

- The switch box is a white box with two buttons on its front.
- A cable runs from the GC to the box and separate cables run from the box to the printer/plotter and to the computer.

c) Press F8 (Return to term index).

d) Press F6 (Plot/replot).

e) Press PAGE.

f) Press F6 (Reprint on Printer/plotter).

- A hard copy of the trace will print out.

g) Wind the paper back to start of trace (Printer Offline then Online when rewound).

- This allows the data to be printed beside the trace and saves paper.

h) Press F8 (Return to term index).

i) Press F5 (Pre/post).

j) Press F5 (Report).

k) Press F2 (Print report).

- The data will print out on the Printer/plotter.

6.3 Transferring data to computer

a) Make sure computer is on (see Section 6.1).

b) Press button B (computer) on switch box.

c) Press F2 (Print report).

- Data will print out on computer screen; a copy of the trace will not be transferred.

d) Press F1 on the computer while XTALK is displayed.

- This will run a series of steps to save the data on diskette.
- Several screens will flash briefly on the screen.
- When the status line again displays:

  "Esc for ATtention, Home to SWitch ¦ Capture to A:DATA.GC ¦ Local"

  the data is saved and a new run can be started.

- Data should be saved after every run.

e) Press Button A (Printer/plotter) on switch box.

- This prevents the trace being accidentally transferred to the computer at the end of the next run.

6.4 Data analysis

At this time, there is both a 'hard' and a 'soft' ASCII copy of the data from the run. This provides a great deal of flexibility for subsequent data analysis. Information on transferring data from the ASCII file through STATGRAPHICS analysis is available upon request from the editor, C. Hawkins at MacMillan Bloedel.

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APPENDIX I

GC PROGRAMS USED IN THE SIVE PROCEDURE

1. GC settings for the analytical method (5 Modified)

a) Section 1 GC control

Heating Program	1	2	3
- Oven Temperature °C	100	190	250
- ISO Time min	2.0	3.0	2.0
- Ramp Rate °C · min^{-1}	30.0	30.0	

FID sensitivity High

Detector Temperature 300

Flow 30 mL · min^{-1}

Carrier gas He

Equilibration time 1.0 min

Total run time 12.0 min

b) Section 2 Timed events

Event		Time
- Attenuation	4	-0.30
- Relay 0	ON	-0.20
- Relay 0	OFF	-0.18
- Relay 1	ON	0.01
- Relay 1	OFF	0.03
- Relay 0	ON	8.00
- Relay 0	OFF	8.02

2. GC settings for the CLEANUP method

a) Section 1 GC control

Heating programs	1
- Oven Temperature °C	250
- ISO Time min	999.9
- Ramp Rate °C • min^{-1}	

FID sensitivity Low

Detector Temperature 300

Flow 30 mL · min^{-1}

Carrier gas He

Equilibration time 1.0 min

Total run time 15.0 min

b) Section 2 Timed events

Event		Time
- Relay 0	ON	-0.20
- Relay 0	OFF	-0.18
- Relay 1	ON	0.01
- Relay 1	OFF	0.03
- Relay 0	ON	15.00
- Relay 0	OFF	15.02

APPENDIX II

COLUMN PACKING

Because the carrier gas passes through the analytical column one way during analyses and the other way during backflush mode, these columns have to be fully packed. This is to prevent the extinction of the flame ionization detector by changes in carrier gas pressure as the machine switches from forward flush to backflush mode. Coldtrapping columns can be packed by the same method but only need to be packed from one end.

1. Analytical column

   a) 180.0 x 0.32 cm stainless steel tubing.

   b) Packed with 80-100 mesh HayeSep Q.

2. Coldtrapping column

   a) 16.0 x 0.32 cm stainless steel tubing.

   b) Packed with 80-100 mesh HayeSep N.

3. Packing method

   a) Cut required length of stainless steel column tubing using a tubing cutter.

   b) Using hand held reamer, open cut ends to full inside diameter of the tubing.

   c) Form the tubing into a "U" shape by gently bending in the middle of the cut length.

      - For the coldtrapping column, do this AFTER it is packed.
      - When packing the coldtrapping column, START by placing a small plug of silanized glass wool in one end of the tubing (see Appendix II, Section 3, k)

   d) Hold the tubing in a clamp on a retort stand.

   e) Attach the packing funnel to one end of the tubing.

   f) Pour a small amount of HayeSep Q (HayeSep N for coldtrap column) into the funnel.

   g) Using a vibrator (vibrating engraving tool), vibrate the column material so that the packing material runs into the column.

      - Keep topping up the funnel until the level of packing material in the funnel does not go down.

h) Remove the remaining packing material from the funnel.

- For the coldtrapping column, NOW insert the second plug of silanized glass wool (Appendix II Section 3, k).
- BEND the coldtrapping column into a "U".

i) Transfer the funnel to the other end of the tubing.

j) Repeat steps in Appendix II Section 3 (f-h).

k) Using the small forked wire tool insert a small plug of silanized glass wool into each end of the tubing.

l) Place a Swagelock connector on each end of the tubing. This applies to both columns.

- Slip a female Swagelock nut over the end of the tubing.
- Slip a rear collar over the tube with the narrower end towards the end of the tube.
- This is the shorter collar of the two.
- Slip a conical collar over the tube with the apex of the cone towards the end of the tube.
- This is the longer collar of the two.
- Attach the nut to a male Swagelock connector and tighten down firmly.
- This will seal the Swagelock connector onto the tubing.
- Repeat Appendix II Section 3 (l) for the other end of the tubing.

m) Bend the tubing into a coil by wrapping tightly around a cylinder of approximately 10 cm diameter.

- This is NOT done for the coldtrapping column.

n) Attach a metal label to the column with the length, diameter, packing material and date of packing inscribed on it.

o) Attach the column to the injector and the detector connectors inside the oven.

p) COLDTRAPPING column MUST be installed so that the air flow is in the SAME DIRECTION as the packing was done.

- Air flow enters at end where LAST wool plug was inserted.

q) Condition the column over night at 250°C with carrier gas running through it.

- See startup procedure (Section 4.2, Appendix I, #2).

r) The column is now ready for use.

APPENDIX III

TROUBLE SHOOTING

1. PP indicator

READY state will not go on and PP is highlighted at top right of screen.

- Switch on Printer/plotter.

2. FLOW indicator

READY state will not go on and FLOW is highlighted at the top centre of screen. There is no carrier gas flow.

- Run a clean up run for at least an hour or until FLOW is turned off.
- If indicator light does NOT go OFF, use bubble flowmeter to ascertain whether there is a flow of carrier gas through the column.

3. Other problems

Other problems and their trouble shooting are described in the Perkin Elmer GC manual.

APPENDIX IV

SAFETY

1. Handling of gas cylinders.

   a) All compressed gas cylinders must be kept upright and securely fastened to the wall or bench.

   b) Do not stand directly in front of regulator pressure gauge while a cylinder is being opened.

   c) Mark cylinders clearly to identify the contents.

   d) Use only approved regulators and hose connectors.

   e) When the equipment is turned off, close all gas cylinder valves tightly at the tank.

   f) Hydrogen (H_2).

      - Non toxic but can cause asphyxiation by excluding air.
      - Forms explosive mixtures with air and O_2.
      - Is a flammable liquified gas.

   g) Helium (He)
      - Non toxic but can cause asphyxiation by excluding air.

2. Water baths

   Always be sure that all boiling water baths have been turned off when leaving the room for more than a few minutes.

3. Flame ionization detector

   Always be sure that the FID has been turned off at the end of a working day (TURN OFF UHP air and H_2).

4. Volatiles

   Acetaldehyde standards should ALWAYS be stored in the fridge. Acetaldehyde is highly volatile and explosive. It is also toxic.

   Do all transfers in the fumehood.